Experimental and Theoretical Study of Lanthanide Complexes Based on Linear and Macrocyclic Polyaminopolycarboxylic Acids with Pyrazolylethyl Arms

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1. INTRODUCTION

Lanthanide complexation chemistry has acquired a large interest and rapid progress over the past several years. In particular, gadolinium (III) complexes, derived of polyaminopolycarboxylic acids, are commonly used as contrast agents (CAs) in Magnetic Resonance Imaging (MRI) [1]. The most commonly Gd-complexes used for this propose are [DTPA(Gd)$(H_2O)]^{2-}$ and $[DOTA(Gd)(H_2O)]^-$ deriving of diethylentriaminepentaacetic and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acids, respectively. More recently, several examples of chelating ligands including heterocyclic rings have been reported. Concretely, pyridine and tetrazole have been studied as isosteric groups of carboxylate moiety in polyaminopolycarboxylic acids [2]. Here we describe the synthesis and relaxometric characterization of two novel chelating ligands **1** and **2** with 3,5-pyrazolylethyl arms (figure 1).

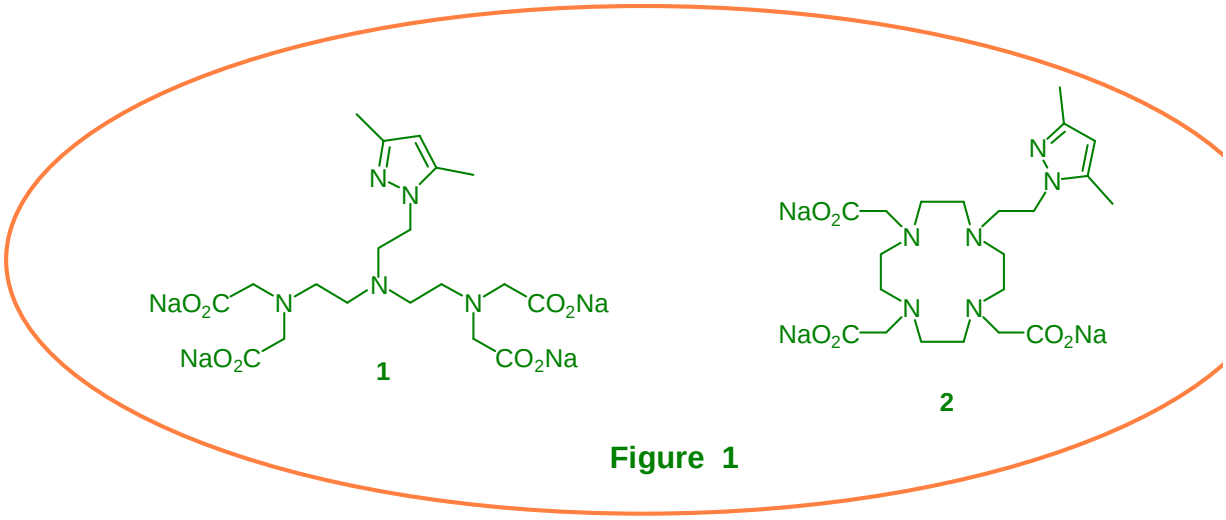

Figure 1

We analyze the effect of including the 3,5-dimethylpyrazolylethyl moiety in the magnetic and complexation properties of the corresponding lanthanide complexes **of 1** and **2**, as compared with Gd (III)-dtpa and Gd(III)-dota.

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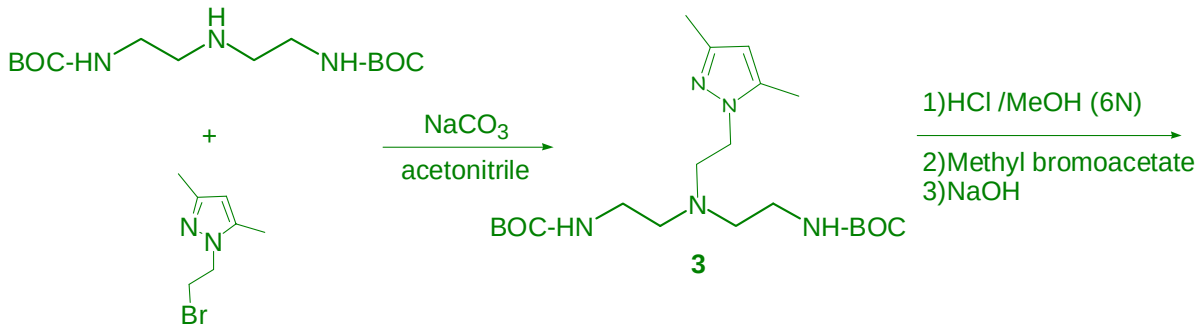
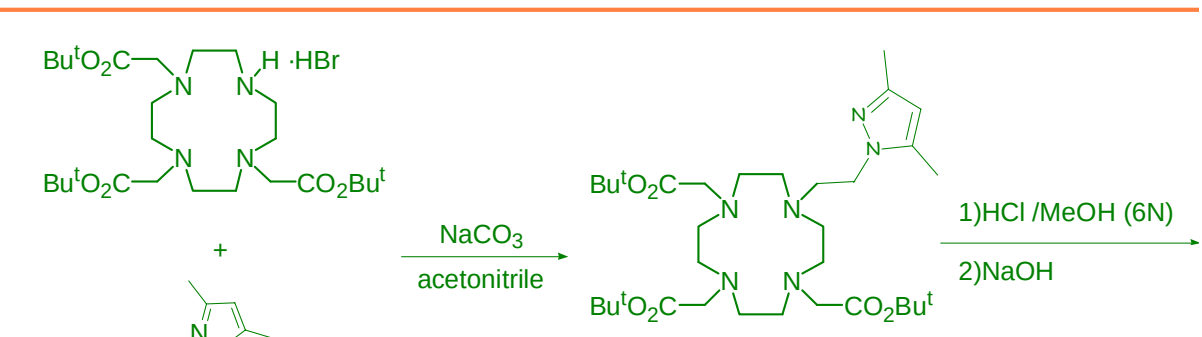
2. RESULTS AND DISCUSSION

Ligands **1** and **2** were prepared starting from *N,N'*-Boc-diethylentriamine [3] and 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic

acid hydrobromide [4], respectively (scheme 1 and 2). The corresponding linear and macrocyclic amines react with 2-bromoethyl-3,5-dimethylpyrazole [5] to give the compounds **3** and **4**. *Tert*-butoxycarbonyl groups of compound **3** were removed in acidic medium and the corresponding amine was treated with methyl bromoacetate. Basic hydrolysis of the obtained methyl aminoester yielded the compound **1** (scheme 1). Analogously, acidic hydrolysis of **4** gave the corresponding acetic acid which was characterized as its trisodium salt **2** (scheme 2).



Scheme 1



Scheme 2

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2.1. Relaxometric Study

Gd(III)-complexes have been synthesized using equimolecular amounts of the corresponding ligands and $GdCl_3 \cdot 6H_2O$ at room temperature for several minutes. They have been characterized by IR-FT (ATR) and ESI-MS (negative ions mode) finding 1:1 stoichiometry.

The efficacy of a potential contrast agent can be evaluated by its proton relaxivity (r_1 and r_2) in aqueous solutions expressed in s^{-1} mM^{-1}

(figure 1). Relaxivity was calculated from equation 1:

$$r_{1(2)} = \Delta\,(1 / T_{1(2)}) / [GdL] \qquad [1]$$

where, for every complexone, Δ is the d
rates ($1 / T_{1(2)}$) of the water protons in p
Gd(III)-complex, and [GdL] the molar co
complex.

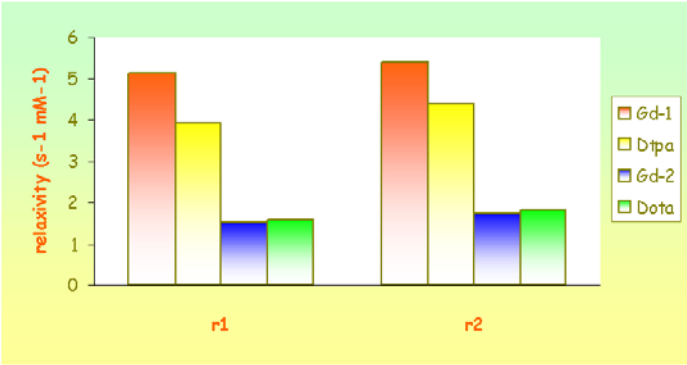

Figure 1. r_1 and r_2 have been determined
Minispec 60 MHz using the inversion recove
Carr Purcell Meiboon Gill (T_2) sequences, r
Model solutions: 100 mM TRIS / HCl (pH ~ 7,
150 mM NaCl and 1 mM complexones or Gd-co

Figure 1 shows r_1 and r_2 values of **Gd-1** and **Gd-2** as compared with Gd-dtpa and Gd-dota. **Gd-1** exhibited a $r_{1(r2)}$ maximum values, even higher than dtpa, while **Gd-2** and dota presented similar relaxivity.

Figure 2 and 3 despite the pH and temperature dependence of r_1 at 60 MHz, respectively.

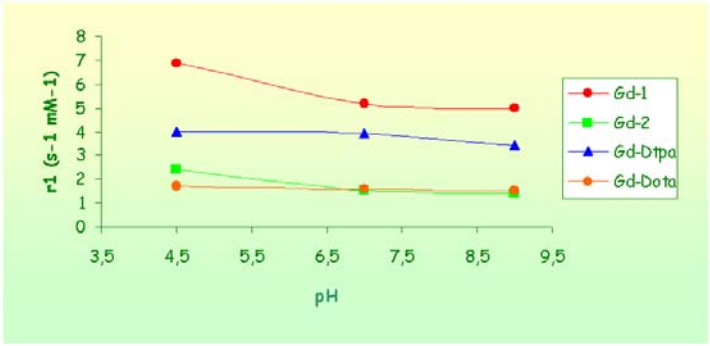

Figure 2. pH dependence of the r1 at 60 MH
T = 37 °C
r_1 of Gd-dtpa and Gd-dota are constant in a
range of 4.5-9

r1 Gd(III)-1 >> r1 Gd(III)-2 pH 4.5

**The macrocyclic complex shown a stron
thermodynamic stability and weak
dissociation at acidic pH.**

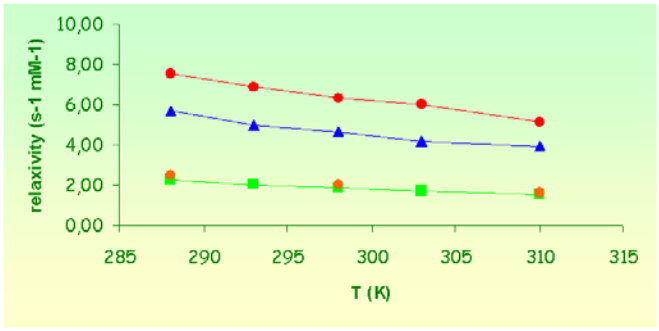

Figure 3. Temperature dependence r1 at 60 MHz and pH ~ 7

Solomon- Bloembergen-Morgan Th

$r_{1(2)} = q / 55.5 (T_M + \tau_M)$

Two limited cases:

$T_M << \tau_M$ $r_{1(2)}$ ↓ T ↓

$T_M >> \tau_M$ $r_{1(2)}$ ↑ T ↓

Considering the Solomon-Bloembergen-Morgan Theory, temperature dependence of r_1 is a qualitative assessment of the τ_M, being $r_{1(2)}$, the longitudinal and transversal relaxivity, q, the hydration number (water molecules in the inner-sphere), T_M the relaxation time of water protons of the water bound to metal center and finally, τ_M the residence time of water in inner-sphere. It was observed an increased of water exchange rate in inner-sphere in Gd(III)-complexes of **1** and **2**.

Hydration number, q, has been determined by *^{17}O NMR* of the corresponding Dysprosium complexes (figure 4).

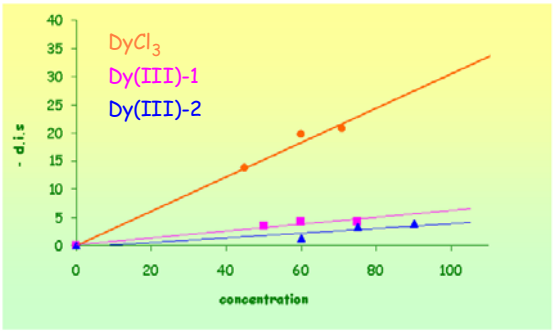

Figure 4. Dysprossium induced shift (d.i.s) vs complex concent

^{17}O-NMR of the corresponding Dy-complex wa solutions allows the approximated determination of number of coordinated water molecules in inner-sphe

- d.i.s = cte [complex] q

Gd(III) complexes of **1** and **2** contain 1.6, 1.0 wate molecules in innersphere per Dy(III) ion, respectively.

Then, hydration numbers (q) of the corresponding complexes have been determined from the slope of the concerned line as compared with the slope for $DyCl_3$ (q = 8) [6]. For $DyCl_3$, **Gd-1** and **Gd-2**, the slope of the lines are 305.4 (r^2 0.98), 62.7 (r^2 0.95) and 39.1 (r^2 0.98) ppm M^{-1}, repectively. Measurements of solutions of $DyCl_3 \cdot 6H_2O$ have been carried out at pH 3.5 while pH of the corresponding Dy-complexes solutions was around of 6.5-7.

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2.2. Theoretical Calculations

Geometry optimizations were performed with Gaussian 98 [7] at the HF/3-21G/CPCM level with the ECP of Dolg et al. (46+4f^7 electrons in the core) [8]. Then, single-point energy calculations were carried out at the density functional theory level (mPW1PW91 functional), using the 6-31+G** basis sets for the ligand.

*** Thermodynamic Stability**

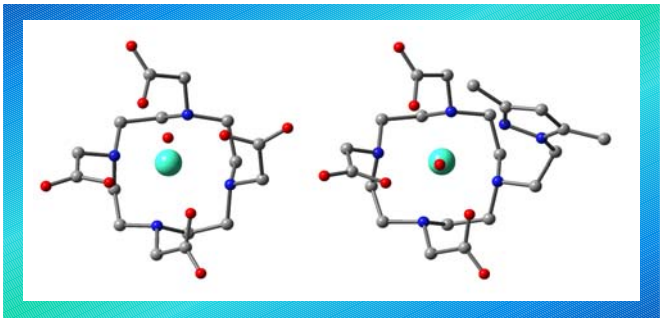

Figure 5. Optimized geometries for the $[Gd\text{-}L(H_2O)]^{n-}$ complexes in aqueous phase.

Mean distances (Å) and SD (in parenthesis) from calculations of $[Gd\text{-}L(H_2O)]^{n-}$ complexes in aqueou

L	Gd-O	Gd-N	Gd-N_{az}
DTPA	2.369 (0.035)	2.689 (0.064)	--
1	2.356 (0.027)	2.678 (0.075)	2.758
DOTA	2.351 (0.025)	2.676 (0.010)	--
2	2.333 (0.017)	2.686 (0.048)	2.775

$\Delta E_{TOTAL}=E_{COMPLEX}-E_{LIGAND}-E_{Gd(III)}$, and ΔE_{REL}, relative to the patern complex (in kcal/mol)

Ligand	ΔE_{TOTAL}	ΔE_{REL}
DTPA	-146.55	0.00
1	-135.74	+10.80
DOTA	-147.80	0.00
2	-135.28	+12.52

The introduction of a pirazolethyl arm results in a shortening of the Gd-O and Gd-N distances for the acyclic system, and the methyl substituent induces steric compression around the water binding site, increasing the Gd-O_W distance and favouring its departure [9]. For the macrocyclic species, the metal center is displaced toward the plane formed by the carboxylate groups, giving rise to a longer Gd-N and shorter Gd-O and Gd-O_W distances. While DOTA complex is slightly more stable than DTPA complex, Gd-**2** appears as weakly less stable than Gd-**1**. The complexes bearing a pyrazolethyl ligand moiety are > 10 kcal/mol less stable than the model, for both acyclic and macrocyclic systems.

*** Kinetic Stability**

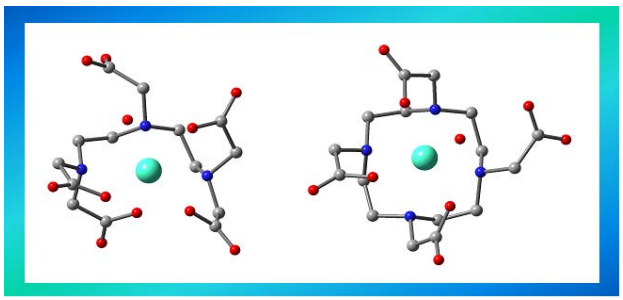

Figure 6. Optimized transition structures for the first step of the dissociation process of the [Gd-L(H_2O)]$^{n-}$ complexes.

Activation energy (in kcal/mol) for the first step o dissociation process of the Gd-DTPA and Gd-D complexes.

Ligand	Gas Phase	Aqueous Phase[a]
DTPA	18.48	12.49
DOTA	30.06	22.26

[a] Transition states in aqueous solution could not b characterized by vibrational analysis due computational limitations.

The kinetic stability of the Gd(III)-complexes plays a critical role in determining the toxicity, and it can be characterized by analyzing exchange reactions with other ions. The computed activation energy values suggest that the first step of the complex dissociation are kinetically more difficult for the macrocyclic system. The results are in agreement with the experimental evidence suggesting a higher kinetic stability of the rigid macrocyclic as compared with the acyclic ligands.

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3. CONCLUSIONS

*We have communicated the synthesis, study of magnetic properties and theoretical calculations of Gadolinium complexes as derived of linear and macrocyclic CAs with 3,5-dimethylethyl pendant.

*Relaxivity (r_1 and r_2) values of those are either higher or similar as compared with the parents compounds, Gd(III)-dtpa and Gd(III)-dota, respectively.

*The 3-methyl substituent on the pyrazolethyl arm induces a higher steric compression around the water binding site for dtpa- than for dota-derivatives, which can accelerate the water exchange process, thus increasing the relaxivity values.

*Activation energy results for the dissociation complex support the higher kinetic stability of the macrocyclic complexes.

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4. REFERENCES

[1] a) Merbach, A. E.; Tóth, E. *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, John Wiley & Sons, Ltd.; England, 2001. b) Gries, H. *Top. Curr. Chem.* **2002**, *221*, 1-235.

[2] a) Cheng, T. H.; Wang, Y. M.; Lee, W. T.; Liu, G. C. *Polyhedron* **2000**, *19*, 2027. b) Aime, S.; Botta, M.; Frullano, L.; Geninatti, S.; Giovenzana, G.; Pagliarin, R.; Palmisano, G.; Sirtori, F. R.; Sisti, M. *J. Med. Chem.* **2000**, *43*, 4017. c) Aime, S.; Gianolio, E.; Corpillo, D.; Cavallotti, C.; Palmisano, G.; Sisti, M.; Giovenzana, G. B.; Pagliarin, R. *Helv. Chim. Acta* **2003**, *86*, 615. d) Aime, S.; Cravotto, G.; Geninatti, S.; Giovenzana, G. B.; Ferrari, M.; Palmisano, G.; Sisti, M. *Tetrahedron Lett.* **2002**, *43*, 783.

[3] Rannard, S. P.; Davis, N. J. *Org. Lett.* **2000**, *2*, 2117.

[4] Li, C.; Wong, W. T. *Tetrahedron,* **2004**, *60*, 5595.

[5] López, P.; Seipelt, C. G.; Merkling, P.; Sturz, L.; Álvarez, J.; Dölle, A.; Zeidler, M. D.; Cerdán, S.; Ballesteros, P.; *Bioorg. Med. Chem.* **1999**, *7*, 517.

[6] a) Cossy, C.; Helm, L.; Powell, D. H.; Merbach, A. E. *New J. Chem.* **1995**, *19*, 27. b) Kowall, T.; Foglia, F.; Helm, L.; Merbach, A. E. *J. Am. Chem. Soc.* **1995**, *117*, 3790. c) Cossy, C.; Barnes, A. C.; Enderby, J. E.; Merbach, A. E. *J. Chem. Phys.* **1989**, *90*, 3254.

[7] Gaussian 98 (Rev. A.11), Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O. *et al.*, Gaussian, Inc., Pittsburgh, PA, **1998**.

[8] Dolg, M.; Stoll, H.; Savin, A.; Preuss, H. *Theor. Chim. Acta* **1989**, *75*, 173.

[9] Laus, S.; Ruloff, R.; Tóth, E.; Merbach, A. *Chem. Eur. J.* **2003**, *9*, 3555.

5. ACKNOWLEDGEMENTS

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